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Immunogenetics in primary sclerosing cholangitis

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Abstract

Purpose of Review: Primary sclerosing cholangitis (PSC) is progressive biliary liver disorder strongly associated with inflammatory bowel disease (PSC-IBD). We summarize the genetics of PSC-IBD and highlight recent findings that further differentiate PSC-IBD as a unique disease.

Recent findings: To date, genome-wide studies have uncovered 23 susceptibility loci for PSC-IBD; the majority of which have been previously reported as risk factors in other immune-mediated disorders. For most candidates, the pathological relationship to PSC-IBD remains largely unknown. Several of candidate genes appear to be liver-related but the large majority relate to immunity and reaffirm that alterations to immune function, trafficking and tolerance are likely to influence susceptibility and presentation of PSC-IBD. Similar to most immune-mediated diseases, the strongest association in PSC-IBD resides within the human leukocyte antigen (HLA) complex and suggests that disease-specific antigens drive pathogenic immune responses. Although genetic predisposition influences disease, genetic determinants account for less than 10% of total disease liability in PSC-IBD, clearly emphasizing the predominant role of environmental factors on disease susceptibility.

Summary: Genetic studies define PSC-IBD as a unique disease to IBD mirroring clinical observations. Most risk loci harbour immune-related genes and disease variants are likely to perturb immune function, tolerance and/or trafficking. Additional studies in patients and novel experimental systems are needed to identify the origin and impact of environmental factors in relation to genetic predisposition in PSC-IBD.

Keywords

primary sclerosing cholangitis, inflammatory bowel disease, genome-wide association studies, autoimmunity, pleiotropy, microbiota

Introduction

Primary sclerosing cholangitis (PSC) is an enigmatic liver disorder marked by biliary infiltration and bile duct strictures leading to recurrent cholestasis, cirrhosis and end-stage liver disease. Characteristic inflammatory and obliterative concentric periductal fibrosis ("onion-skinning") can affect both intrahepatic (small) and extrahepatic (large) bile and in 50-80% of cases clearly associates with a distinct form of inflammatory bowel disease (PSC-IBD) that features rectal sparing, backwash ileitis and right-sided predominance [1]. In line with clinical observations, genetic studies show that PSC-IBD shares limited overlap with IBD (~50%) and is driven by a unique set of genetic determinants compared to ulcerative colitis (UC) [2,3**,4**]. Whilst PSC in the absence of IBD may reflect true endotypes – subtypes of a condition defined by distinct pathological mechanisms – all patients may in fact experience mild colitis which is simply undetectable by current techniques.

In early disease, patients with PSC-IBD typically present with elevated serum alkaline phosphatase (ALP) activity, often in the context of asymptomatic cholangitis and at later stages, fatigue, pruritus, splenomegaly, hepatomegaly and jaundice are common [5]. PSC-IBD carries an elevated risk of cancer and 10-20% of patients develop some form of hepatobiliary or colorectal malignancy [6,7*]. Incidence (0.9-1.3 per 100,000) and prevalence (8.5-13.6 per 100,000) of disease is increasing and varies geographically emphasizing that environmental factors significantly affect disease susceptibility [8,9]. Onset is generally between 30-40 years of age and unlike most autoimmune disorders, PSC-IBD affects males more frequently than females (2:1). Heritability is comparable to other complex immune-mediated disorders as first-degree relatives are 10-times more likely to develop PSC-IBD compared to unrelated individuals [10]. Effective

medical treatments are a critical unmet clinical need as the basic pathophysiology of PSC-IBD is poorly understood and liver transplantation remains the only means of managing disease progression [11].

Genetic studies in PSC-IBD

Over the last decade, genome-wide association studies (GWAS) and targeted genotyping arrays have identified dozens of genes linked to the development (susceptibility genes) and phenotype (modifier genes) of PSC-IBD [2,3**,4**,12-16]. To date, these large-scale case-control investigations have uncovered a total of 23 risk loci of genome-wide significance (Table 1), most notably a striking human leukocyte antigen (HLA) association that closely resembles prototypical autoimmune disorders such as rheumatoid arthritis (RA), type 1 diabetes (T1D) and coeliac disease (CD) [17]. Weaker associations exist for the non-HLA loci and the majority of genes within these regions relate to immune pathways which all have been previously identified as risk loci in other immune-related diseases [18], a genetic term known as pleiotropy [19].

Non-HLA loci in PSC-IBD have small effect sizes that overlap with other immune-mediated conditions

As the number of gene associations in PSC-IBD expands, the translation of these discoveries into disease biology remains challenging as none of the candidate genes harboured within these regions confer independent causality and considerable genetic heterogeneity exists in complex

disease [20]. For example, *ASAP2* and *NFKB1* associations were recently identified for PSC-IBD [16] suggesting ASAP2-mediated phagocytosis by innate cells [21*] and the myriad of NFK1B-mediated processes in B and T cells have a role in pathogenesis [22]. Likewise, associations for BACH2 were previously reported [2] and recent data showing that BACH2 regulates the transition of lymphoid cells into memory lineages [23*,24*] similarly implies contributions of adaptive immunity to PSC-IBD. Although appealing, it is speculative to assign a precise pathogenic role to any of the gene associations in PSC-IBD since none of the loci point towards an obvious biological process, which differs from the primary biliary cholangitis (PBC), where disease-specific auto-antibodies to mitochondrial antigens (anti-mitochondrial antibodies; AMA) and associations affecting multiple pathways of immune tolerance [25] more clearly delineate PBC as autoimmune. Moreover, in contrast to monogenic disorders where a strong penetrance/causality exists, variants associated with complex disease have small effect sizes exemplified by the fact healthy individuals carrying multiple risk variants never develop disease.

In lieu of causality, several important concepts have emerged from genetic studies in PSC-IBD that have been useful for the validation of clinical observations and prioritization of future research. First, cross-disease studies show that PSC-IBD is genetically distinct to IBD as less than half of the risk loci overlap between diseases and most of these associations are stronger in PSC-IBD than in IBD [2,3**,4**]. As the co-morbidity of IBD with PSC is reported in up to 80% of cases, the restricted overlap between risk loci is surprising and could be interpreted to suggest that the shared associations are key drivers of IBD or that PSC-IBD is indeed a unique disease that manifests both colonic and biliary phenotypes. Neither scenario is mutual exclusive however clinical observations and recent genetic data supports the latter explanation whereby PSC-IBD is distinct to IBD but shares a certain degree of genetic similarity [2,3**,4**].

Second, consistent with prevailing notions that PSC-IBD is autoimmune in nature, risk loci for PSC-IBD show considerable genetic overlap with archetypical autoimmune disorders such CD, T1D, multiple sclerosis and ankylosing spondylitis [2,3**,4**]. Again, genetic studies mirror clinical practice as patients with PSC-IBD are known to present with a higher co-incidence of immune-mediated diseases [26,27], implying that the pleiotropic loci in PSC-IBD may belong to a general pool of predisposing autoimmune risk factors. These finding also support a model of disease alongside the HLA association whereby currently unknown disease-specific antigens and auto-antibodies trigger or worsen progression PSC-IBD prior to the development of cholestasis and bile-acid toxicity. Lastly, several PSC-IBD loci, including *BACH2*, *CD226*, *IL-2*/*IL-21* and *MST1*, are highly pleiotropic, associating with UC, Crohn's disease, T1D, C1D, RA and alopecia areata [2,3**,4**]. The pleiotropism of these loci are likely indicative of common perturbations that promote the onset of immune-mediated conditions. Hence, insights gained from studying any of these diseases, particular the pathways relating to shared loci, may yield relevant considerations for disease biology and etiologies in PSC-IBD.

Collectively, the genetic risk in PSC-IBD is estimated to account for less than 10% of total disease liability and comparable to other complex, polygenic immune-mediated diseases (ex. 13.5% for Crohn's disease, 7.5% for UC) [18]. Better assessment of genetic risk will require larger GWAS cohorts and the application of whole exome and genome sequencing technologies to identify rare variants of potentially large effect size given that genome-wide surveys are biased towards the detection of common alleles carried by greater than 1 – 5% of the general population. The modest contribution of GWAS loci to PSC-IBD emphasizes that non-genetic determinants of

unknown origin, presumably individual diet, toxin exposure, infection and microbiota, underpin disease susceptibility and phenotype on a backdrop of genetic predisposition.

HLA association, TCR sequencing analysis and auto-antibody profiling points towards disease-specific antigenic triggers in PSC-IBD

HLA associations are implicated in nearly every immune-mediated disorder but their relevance to disease biology has proven difficult to reconcile. In some instances such as T1D and CD, HLA associations have been shown to correlate with the presentation of tissue-specific antigens that elicit the activation and proliferation of pathogenic T cells [10,28,29]. For PSC-IBD, HLA associations reported by GWAS confirm earlier reports of such associations [28,29] and refined mapping of the HLA locus has identified HLA-B8 (HLA class I) and HLA-DRB1 (HLA class II) as the predominant risk alleles in PSC-IBD [17]. HLA-B8 and HLA-DRB1 are linked to many autoimmune conditions which supports the notion that breakdown of immune tolerance leads to immune-mediated hepatobiliary destruction [30].

The robust HLA association and chronic infiltration of immune cells in areas of hepatobiliary injury suggests disease-specific antigens are responsible for driving pathogenesis in PSC-IBD. Consistent with this hypothesis, T-cell receptor (TCR) sequencing analysis of the liver-infiltrating T cells in PSC-IBD explants has detected evidence of antigen-driven clonal expansion and presence of disease-associated clonotypes [31**]. TCR sequencing analysis has also demonstrated that memory T cells of common clonal origin are found at a higher frequency in paired gut and liver samples of PSC-IBD patients compared to controls [32**], implying

antigenic triggers (microbial, dietary or endogenous) may originate from the gut and enter the liver via the portal circulation to initiate or perpetuate immune activation [7*,33**].

In several immune-mediated diseases such as CD, RA and PBC, disease-specific auto-antibodies serve as clinical diagnostic biomarkers and have been used to identify disease-relevant antigens and dissect the role of specific HLA associations in pathogenesis [34-36]. For PSC, auto-antibodies to biliary and colonic antigens as well as neutrophil granulocytes (perinuclear anti-neutrophil cytoplasmic antibodies; pANCA) have been reported in patient sera however none of these reactivities demonstrate robust validation or disease specificity [37,38]. Antibody profiling of liver-infiltrating B cells from PSC-IBD explants has identified several novel reactivities of potential relevance however these findings require additional validation in large multi-centre study cohorts [39**]. Importantly, such studies using high-throughput technologies may be useful for identifying additional disease-specific auto-antibodies for PSC-IBD and alongside TCR cloning and HLA antigen-elution, aid the characterization of key molecules applicable for the development of tolerizing immunotherapy [40*].

Host genetic factors affect the composition of gut microbiota

Although the breakdown of immune tolerance and activation of self-reactivity are probable mechanisms of disease in PSC-IBD, understanding how environmental factors drive the development of autoimmunity is poorly recognized. As gut commensals are known to regulate immune function and bile acid metabolism [41,42], the dysbiosis detected in both fecal and mucosal samples of patients with PSC-IBD may be indicative of an environmental risk for the

development of autoimmunity [33,43-44,45*46**]. Intriguingly, several recent studies in both humans [47**,48**] and mice [49-51] indicate host genetics, particularly the variability associated with immune genes, influences the composition of gut microbiota which implies that variants associated with disease may predispose individuals to gut dysbiosis that promotes autoimmunity. For PSC-IBD, variants of the suspected risk gene *FUT2* are known to alter the biliary microbiome in PSC-IBD [14] and it remains to be seen if the dysbiosis in PSC-IBD is caused by genetic factors or by non-genetic determinants such as diet. Nevertheless, these findings raise the possibility that gut dysbiosis in the context of genetic predisposition may sway the colonization of gut mucosa from healthy to pathogenic in patients with PSC-IBD. Given that gut commensals are sensitive to diet and medical interventions [52**,53**], targeted alterations of the gut microbiome in conjunction with fecal microbiota transplant from healthy individuals may lessen or ameliorate disease.

Certain functional characteristics of gut commensals are expected to underlie individual host-microbe interactions therefore future studies utilizing meta-genomics with traditional 16s RNA sequencing may better detect the subtle functional differences that influence pathogenesis. Caution should be taken when using stool samples for the assessment of disease-associated microbes as fecal microbiota represent a mixture of mucosal microbes from the colon and lumen [54] and microbes enriched in stool may not be present or pathogenic at the active sites of liver and gut inflammation. As the intimate interactions between the microbiome and mucosal surfaces may be absent in stool, efforts to study the most appropriate tissue from PSC-IBD patients, genetically similar pathologies (IBD, Crohn’s disease) and other chronic autoimmune and metabolic liver diseases (PBC, autoimmune hepatitis, alcoholic liver disease, non-alcoholic steatotic hepatitis) should be prioritized when possible.

Conclusion

A total of 23 susceptibility loci have now been identified for PSC-IBD and larger GWAS using well-phenotyped cohorts will further define the genetic architecture of PSC-IBD. From these insights, effects of specific variants can be mechanistically evaluated in experimental models which should lead to a greater understanding of the fundamental pathways underlying disease susceptibility and severity. As genome-wide surveys are limited to detect common variants, whole exome and genome sequencing studies as well as meta-data and epigenetic analyses will be important approaches for studying familial presentations and dissecting PSC endotypes. Emerging evidence suggests that genetic variability of the host, particularly in genes relating to immune function, influence gut microbiota indicating that a better understanding of this association in PSC-IBD may unlock tractable therapeutic targets.

Immunogenetic discoveries whilst exciting have continued to however be a challenge to translate into therapeutic advances. This is because disease manifestation and the impact for a patient go far beyond the triggers of disease, and capture both the consequence of tissue damage, and the response to injury, alongside a presumed variation in environmental triggers. What determines a patient's clinical course is therefore multi-faceted and dynamic. Genetic association studies identify pathways that contribute to risk, but the singular impact is usually unclear, with composite risk difficult to measure, and individual patient presentation in the absence of genetic risk frequent. This is further relevant to cholestatic liver diseases where disease cause and disease consequence need not parallel, and disease treatments that traditionally have focused on the consequence of cholestasis have proved effective in some settings, whilst clearly not tackling fundamental disease triggers. New therapies may therefore be identified by capturing broad

pathways to disease and its response, but the hope that individual gene loci become therapeutic targets is less probable.

Key points

1. PSC-IBD is clinically and genetically distinct from IBD.

2. HLA associations are the strongest risk loci in PSC-IBD and point towards disease-specific antigenic triggers.

3. Non-HLA associations for PSC-IBD are pleiotropic and predominantly correspond to immune-related genes.

4. Risk loci in PSC-IBD may affect the composition of gut commensals that influence development and severity of disease.

5. Translating genetic discoveries into relevant biology and therapeutics for complex diseases such as PSC-IBD remains challenging.

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Tables

Table 1. Risk loci in PSC-IBD.

Study	Year	Study design	Cohort ethnicity	Patients (n)	Controls (n)	Risk loci ($P < 5 \times 10^{-8}$)
Karlsen *et al*. [12]	2010	GWAS	Northern European	285	298	*HLA*
Melum *et al*. [13]	2011	GWAS	Northern European Central European European American	1740	5136	*BCL211, MST1, HLA,*
Srivastava *et al*. [15]	2012	targeted genotyping	Northern European	992	5162	*IL2RA, MST1*
Folseraas *et al*. [14]	2012	GWAS	Northern European Central European European American	1936	6470	*TNFRSF14/MMEL1*
Ellinghaus *et al*. [16]	2013	GWAS	Northern European Central European	1401	5530	*GPR35, TCF4*
Liu *et al*. [2]	2013	targeted comparative analysis (Immunochip)	Pan-European	3789	25,079	*BACH2, CD226, CD28, HDAC7, HLA, IL2/IL2, IL2RA, MST1, PRKD2/STRN4, PSMG1, TNFRSF14/MMEL1, SH2B3/ATXN2, SIK2*
Ellinghaus *et al*. [3]	2016	targeted comparative analysis (Immunochip)	Pan-European	3,408	34,213	*ASAP2, NFKB1, RIC8B, LOC107984859*
Ji *et al*. [4]	2016	GWAS	Northern European European American African Han Chinese Japanese	4,796	19,955	*CCDC88B, CLEC16A, FOXP1, UBASH3A*